Neural and general splicing factors control self-renewal, neural survival and differentiation

Grant Award Details

Neural and general splicing factors control self-renewal, neural survival and differentiation

Grant Type: Basic Biology III

Grant Number: RB3-05009

Project Objective: The broad goals of this project are to elucidate the role of alternative splicing in controlling pluripotency, stem cell fate decisions towards the neuronal lineage, and neuronal survival. The role of alternative splicing in neuronal pathology of ALS patients has become a more recent focus.

Investigator:

Name:	Eugene Yeo
Institution:	University of California, San Diego
Type:	PI

Disease Focus: Amyotrophic Lateral Sclerosis, Neurological Disorders, Dementia

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: iPS Cell

Award Value: $1,287,619

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Grant Application Details

Application Title: Neural and general splicing factors control self-renewal, neural survival and differentiation

Public Abstract: Human embryonic and patient-specific induced pluripotent stem cells have the remarkable capacity to differentiate into many cell-types, including neurons, thus enabling the modeling of human neurological diseases in vitro, and permit the screening of molecules to correct diseases. Maintaining the pluripotent state of the stem cell, directing the stem cell towards a neuronal lineage, keeping the neuronal progenitor and stem cells alive - these are all maintained by thousands of different proteins in the cell at these different "stages". Thus the levels and types of proteins are highly controlled by gene regulatory mechanisms.

Genes produce pre-messenger RNA (mRNA) transcripts in the nucleus, which undergo a process of refinement called splicing, whereby long (1,000-100,000 bases) stretches of nucleotides are excised, and much shorter pieces (150 bases) are ligated together to form mature messenger RNA to eventually make proteins in the cytoplasm. Strikingly, some pieces of RNA are used in a particular cell-type, but not another, in a process called "alternative splicing". This is the most prevalent form of generating transcriptome diversity in the human genome, and is important for pushing cells from one state to another i.e. stem cells to neurons, maintaining a cell state i.e. keeping a stem cell pluripotent, or a neuron alive and functioning. Alternative splicing is highly controlled by the recognition of even smaller stretches (6-10 bases) of RNA binding sites) by proteins that bind directly to RNA called splicing factors.

The goal of the proposed research is to produce a regulatory map of where these splicing factors bind within pre-mRNAs across the entire human genome with unprecedented resolution using a high-throughput biochemical strategy. Furthermore, using advanced genomic technologies, we will deduce what happens to splicing when these factors do not bind to their binding sites. Finally, using molecular and imaging methods, we will analyze what happens to survival of stem and neuronal cells when these factors are depleted or over-expressed, and if stem cells are induced to make neurons if the levels of these factors are altered. Completion of the proposed research is expected to transform our understanding of the regulatory mechanisms underlying transcriptome complexity important for neurological disease modeling, especially human neurodegeneration, and stem cell biology. In turn, this will facilitate more accurate comparisons of diseased states of neurons from stem-cell models of Amyotrophic Lateral Sclerosis (ALS), Myotonic Dystropy, Spinal Muscular Atrophy (SMA), Parkinson's and Alzheimer's to identify mis-spliced genes and the splicing factors responsible for therapeutic intervention.

Statement of Benefit to California: Our research provides the foundation for decoding the mechanisms that control the transcriptome complexity of stem cells and neurons derived from stem cells. Our work has direct application in the design of novel strategies to understand the impact of splicing factor misregulation, or mutations within the binding sites for these splicing factors in neurological diseases that heavily impact Californians, such as Amyotrophic Lateral Sclerosis (ALS), Myotonic Dystropy, Spinal Muscular Atrophy (SMA), Parkinson's and Alzheimer's. Our research has and will continue to serve as a basis for understanding deviations from "normal" stem and neuronal cells, enabling us to make inroards to understanding neurological disease modeling using neurons differentiated from reprogammed patient-specific lines. Such disease modeling will have great potential for California health care patients, pharmaceutical and biotechnology industries in terms of improved human models for drug discovery and toxicology testing. Our improved knowledge base will support our efforts as well as other Californian researchers to study stem cell models of neurological disease and regenerative medicine, and for the design of new diagnostics and treatments, thereby maintaining California's position as a leader in clinical and biomedical research.

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